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RESPONSE UNDER 37. CFR 1.116
EXPEDITED PROCEDURE
EXAMINING GROUP 1631

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2-28-02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : **Confirmation No. 6012**

Edwin Southern

Serial No. 09/422,804 : Group Art Unit 1631

Filed October 22, 1999 : Examiner A. Marschel

ANALYSING POLYNUCLEOTIDE SEQUENCES

THE COMMISSIONER IS AUTHORIZED TO CHARGE ANY DEFICIENCY IN THE FEE FOR THIS PAPER TO DEPOSIT ACCOUNT NO. 23-0975.

REQUEST FOR RECONSIDERATION

Assistant Commissioner for Patents,
Washington, D.C.

Sir:

This is responsive to the Official Action dated November 23, 2001, which constitutes a final action.

The Applicant expresses his appreciation for the Examiner's indication that claims 17-77, 79-94 and 96-99 are allowed. Claims 78 and 95 were rejected on the basis of new matter. This ground of rejection is respectfully traversed.

Claims 78 and 95 are supported in the specification as originally filed.

Regarding claim 78, the recitation for a 25 micrometer resolution for a text detection device is supported in the original specification at page 16, line 6 and lines 13-14. A copy of this page showing the support in highlighting is attached hereto.

Regarding claim 95, the recitation for a 5 millimeter wide orthogonal strip is supported in the original specification at page 13, line 10. See also page 28, line 34 to page 29, line 2.

In view of the foregoing, it is respectfully submitted that claims 78 and 95 are supported in the specification as originally filed, and that the rejection under 35 U.S.C. 112, first paragraph should be withdrawn.

If the Examiner has any questions, or if the Examiner is not in agreement, the Examiner is invited to contact the undersigned representative at the telephone number listed below in order to discuss additional actions to expedite allowance of the application.

Respectfully submitted,

Edwin Southern

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February 25, 2002

probe material that needs to be used to achieve a given signal can be much lower.

Autoradiography, especially with ^{32}P causes image degradation which may be a limiting factor determining resolution; the limit for silver halide films is around 25 microns. Obviously some direct detection system would be better. Fluorescent probes are envisaged; given the high concentration of the target oligonucleotides, the low sensitivity of fluorescence may not be a problem.

We have considerable experience of scanning autoradiographic images with a digitising scanner. Our present design is capable of resolution down to 25 microns, which could readily be extended down to less than present application, depending on the quality of the hybridisation reaction, and how good it is at distinguishing absence of a sequence from the presence of one or more. Devices for measuring astronomical plates have an accuracy around 1 μ. Scan speeds are such that a matrix of several million cells can be scanned in a few minutes. Software for the analysis of the data is straight-forward, though the large data sets need a fast computer.

Experiments presented below demonstrate the feasibility of the claims.

Commercially available microscope slides (BDH Super Premium 76 x 26 x 1 mm) were used as supports. These were derivatised with a long aliphatic linker that can withstand the conditions used for the

stripes of the array. Hybridisation is seen at the intersections between oligonucleotide stripes and stripes of test sequence where there is homology between them.

Where sequence variations are known, an advantage of using this technique is that many different mutations can be probed simultaneously by laying down stripes corresponding to each allelic variant. With a density of one oligonucleotide per mm, and one "individual" per 5 mm, it should be possible to analyse 2000 loci on a plate 100 mm square. Such a high density of information, where the oligonucleotides do identify specific alleles, is not available by other techniques.

6. PROBES, HYBRIDISATION AND DETECTION

The yield of oligonucleotides synthesised on microporous glass is about 30 µmol/g. A patch of this material 1 micron thick by 10 microns square would hold $\sim 3 \times 10^{-12}$ µmol, equivalent to about 2 g of human

method we have developed and confirmed by experiments.

Large arrays of oligonucleotides as sequence reading tools.

We have shown that oligonucleotides can be synthesised in small patches in precisely determined positions by one of two methods: by delivering the precursors through the pen of a pen-plotter, or by masking areas with silicone rubber. It is obvious how a pen plotter could be adapted to synthesise large arrays with a different sequence in each position. For some applications the array should be a predetermined, limited set; for other applications, the array should comprise every sequence of a predetermined length. The masking method can be used for the latter by laying down the precursors in a mask which produces intersecting lines. There are many ways in which this can be done and we give one example for illustration:

1. The first four bases, A, C, G, T, are laid in four broad stripes on a square plate.
2. The second set is laid down in four stripes equal in width to the first, and orthogonal to them. The array is now composed of all sixteen dinucleotides.
3. The third and fourth layers are laid down in four sets of four stripes one quarter the width of the first stripes. Each set of four narrow stripes runs within one of the broader stripes. The array is now composed of all 256 tetranuclectides.
4. The process is repeated, each time laying down two layers with stripes which are one quarter the width of the previous two layers. Each layer added increases the length of the oligonucleotides by one base, and the number of different oligonucleotide sequences by a factor of four.

The dimensions of such arrays are determined by the width of the stripes. The narrowest stripe we

have laid is 1mm, but this is clearly not the lowest limit.

There are useful applications for arrays in which part of the sequence is predetermined and part made up of all possible sequences. For example:

Characterising mRNA populations.

Most mRNAs in higher eukaryotes have the sequence AAUAAA close to the 3' end. The array used to analyse mRNAs would have this sequence all over the plate. To analyse a mRNA population it would be hybridised to an array composed of all sequences of the type $N_m AATAAAN_n$. For m + n = 8, which should be enough to give a unique oligonucleotide address to most of the several thousand mRNAs that is estimated to be present in a source such as a mammalian cell, the array would be 256 elements square. The 256 x 256 elements would be laid on the AATAAA using the masking method described above. With stripes of around 1mm, the array would be ca. 256mm square.

This analysis would measure the complexity of the mRNA population and could be used as a basis for comparing populations from different cell types. The advantage of this approach is that the differences in the hybridisation pattern would provide the sequence of oligonucleotides that could be used as probes to isolate all the mRNAs that differed in the populations.

Sequence determination.

To extend the idea to determine unkown sequences, using an array composed of all possible oligonucleotides of a chosen length, requires larger arrays than we have synthesised to date. However, it is possible to scale down the size of spot and scale up the numbers to those required by extending the methods we have developed and tested on small arrays. Our experience shows that the method is much simpler in operation than the gel based methods.